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# Selective alkali metal binding of valinomycin by electrospray ionization mass spectometry

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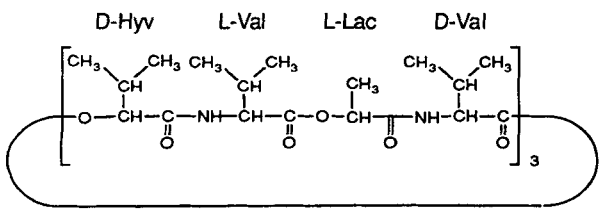
**Selective binding of alkali metal ions by the ionophore antibiotic valinomycin in alcoholic media was detected by electrospray ionization mass spectrometry (ESI-MS). The relative cation affinity for valinomycin is in the order Rb+ > Cs+ > K+ ≫ Na+ in both methanol and ethanol. With a 1:1:1 mixture of valinomycin and synthetic mimics 18-crown-6 and [2,2,2]-cryptate (methanol, excess KOAc) valinomycin showed much higher affinity than the other ionophores. This study has shown that the potential of ESI-MS as a very useful tool for research involving metal binding is great. Problems with sodium ion interference and quantitation are discussed.**

## INTRODUCTION

Valinomycin **1** is a naturally-occurring ionophore antibiotic produced by microorganisms. Its antibiotic nature arises because it promotes changes in cellular ion content and membrane function, thus interfering with the normal working of the cell. A significant feature of this cyclodepsipeptide structure, a cyclic compound composed of alternating α-amino acids and α-hydroxy acids, is that it shows remarkably specific alkali-cation binding.[1] This unique property influences the transport of alkali metal cations through natural and artificial membranes.




**1**

Mass spectrometric techniques have been used previously to study host-guest chemistry such as the complexation of crown ethers and alkali metal ions. Studies based on liquid matrixes (e.g. FAB-MS) showed selectivities similar to solution results obtained by other techniques.[2] Very recently, gas-phase size selectivities of crown ethers have been investigated by a kinetic method.[3] In the gas-phase, different orders of selectivities were observed from solutions of the same complexes.

Electrospray ionization mass spectrometry (ESI-MS) is a new technique that has revolutionized the mass measurement of biomolecules.[4] It usually yields molecular ions with very little fragmentation, implying that deposition of energy into the analyte species is low. In general, preformed ions are required. Since the sample solution is directly injected into the instrument, studies of solution chemistry by mass spectrometry becomes possible. Recent studies of aqueous solutions of metal salts have revealed that the fundamental principle governing electrospray mass spectrometry appears to be solution chemistry.[5] When ions that were identical in charge and similar in type were selected for comparison, quantitative correlations between electrospray responses and calculated equilibrium solution concentrations were observed. Since in these experiments the ions experience very similar electrospray-related processes, such effects on the responses were cancelled. Studies on noncovalent receptor-ligand complexes of FKBP with FK506 or rapamycin showed that the observed peak intensities were in agreement with solution Kd values.[6] We have previously studied the relative binding of 18-crown-6 to alkali metals by ESI-MS. Several instrumental parameters such as repeller voltage, electrospray needle voltage and flow rate affect overall response dramatically, however, good relative responses are maintained under varied operating conditions.

Selective alkali metal ion binding of valinomycin has been studied using many different methods

including fluorescent probes,[7] microcalorimetry,[8] electrochemical reduction[9] and spectroscopy.[10] Recently, mass spectrometry has been used to study the dynamics of the collision-activated decomposition (CAD) of protonated valinomycin.[11] We have been exploring applications of ESI-MS to organic chemistry.[12] Herein we report the direct detection of selective alkali cation binding of valinomycin using this technique.

We have determined the relative alkali metal cation affinity of valinomycin by ESI-MS in alcoholic solutions. The relative potassium binding abilities of valinomycin **1** with synthetic ionophores 18-crown-6 **2** and [2,2,2]-cryptate **3** were also studied.

## RESULTS AND DISCUSSION

Selective alkali metal ion binding by valinomycin **1** has been studied by many analytical methods.[13] All methods show that **1** has the highest selectivity for $Rb^+$. However, different techniques give different relative selectivities for $Na^+$, $K^+$, $Rb^+$ and $Cs^+$.[1a] In our study, we conducted experiments in both methanol and ethanol since binding constants are known in these solvents. When valinomycin was added to a cocktail of metal ions ($Na^+$, $K^+$, $Rb^+$ and $Cs^+$) in methanol, the ESI-MS spectrum shows each of the peptide-metal complexes (Fig 1). Table 1 reports the relative intensities of the metal complexes from ESI-MS in methanol and stability constants from literature.[13] Good agreement with log K was observed although only qualitative agreement with K was found.

Similar experiments were done in ethanol in the presence of two more guests $Li^+$ and $Ca^{++}$ (a total of 6 metal ions). Comparison between the relative intensities of the complexes and their solution stability constants is shown in Table 2.

Since selective potassium binding over sodium is most interesting from a biological point of view, we measured the relative cation affinities of **1** with $Na^+$ and $K^+$. With different concentrations of ligand **1** and metal salts, the relative intensities of the $Na^+$ complex and $K^+$ complex are 1:5.5 and 1:5.7 respectively.[14]

In order to compare ESI-MS results in other systems

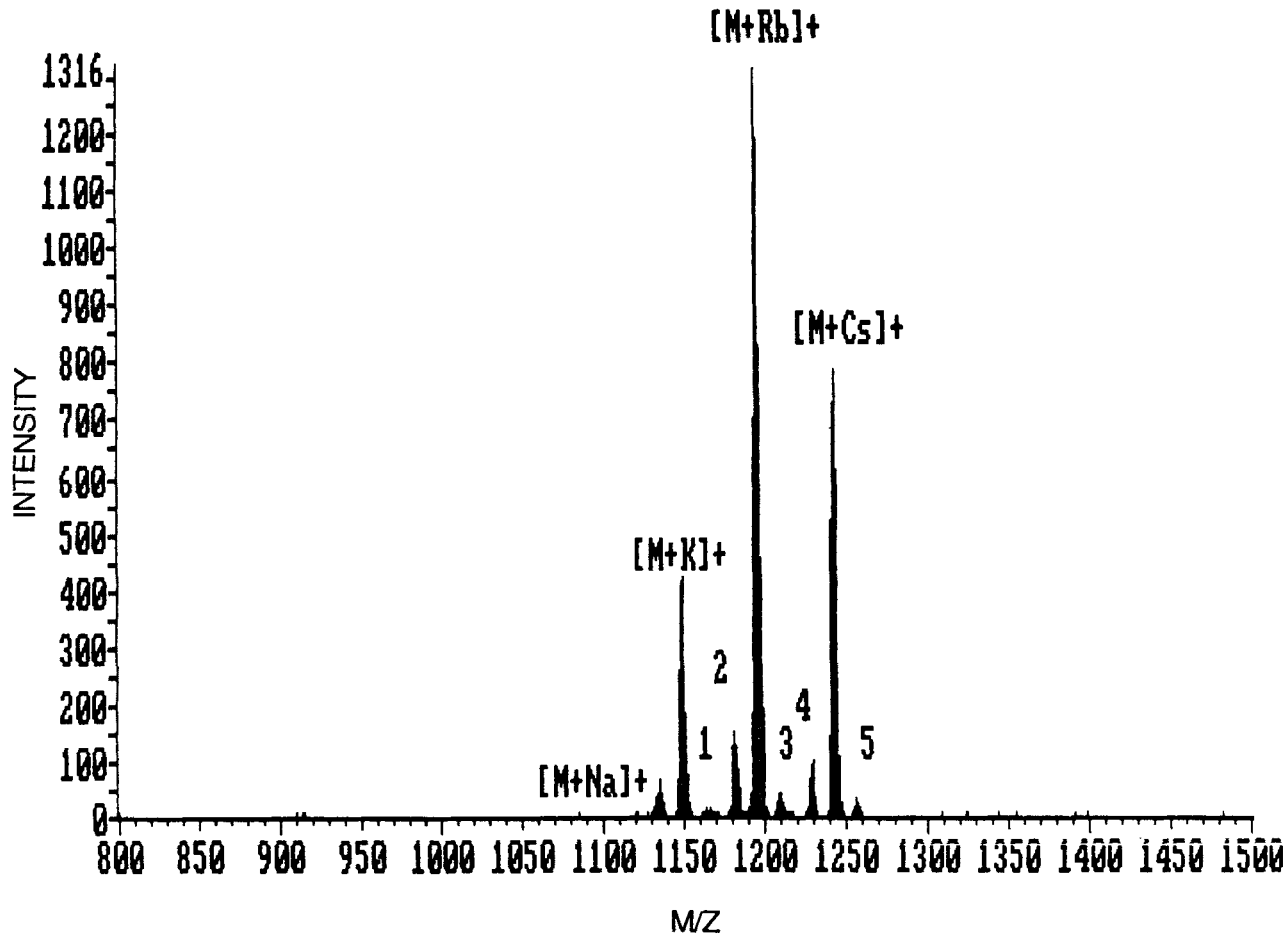



**Figure 1** ESI-MS spectrum of valinomycin **1** in the presence of excess acetates of $Na^+$, $K^+$, $Rb^+$ and $Cs^+$ (metal/**1** ratio is 50:50:50:50:1). M stands for valinomycin **1**. Peak assignments are 1. $[M + Na + CH_3OH]^+$, 2. $[M + K + CH_3OH]^+$, 3. $[M + K + HOAc]^+$, 4. $[M + Rb + CH_3OH]^+$, 5. $[M + Rb + HOAc]^+$.

that have been extensively studied in the gas phase and in solution, similar studies of the relative binding of $K^+$ with valinomycin and synthetic ionophores 18-crown-6 **2** and [2,2,2]-cryptate **3** were done. A mixture of valinomycin, 18-crown-6 **2** [2,2,2]-cryptate **3** and KOAc in methanol was measured by ESI-MS (Fig 2). Table 3 gives the relative intensities of three

**Table 1** Comparison between the relative intensities from ESI-MS and solution stability constants of valinomycin-alkali metal ion complexes in methanol

| | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ |
|---|---|---|---|---|
| Log K[a] | 0.67 | 4.90 | 5.26 | 4.41 |
| Rel. Log K | 1 | 7.31 | 7.85 | 6.58 |
| K | 4.68 | 79433 | 181970 | 25703 |
| Rel. K | 1 | 16972 | 38882 | 5492 |
| Rel. Intensity[b] | 1 | 6.4 | 19.9 | 11.9 |
| Rel. Intensity[c] | 1 | 5.8 | 13.6 | 7.4 |

[a] Measured by a spectrophotometric method.[14]
[b] The concentrations of metal ions and **1** are $2.62 \times 10^{-4}$ M and $5.24 \times 10^{-6}$ M. ESI-MS parameters are same as in the experimental section except: needle voltage 2.30 kV; ES chamber temperature 47 °C.
[c] Same conditions as [b] but intensities are the sum of all ions derived from complexation of the same metal.

**Table 2** Comparison between the relative intensities from ESI-MS and solution stability constants of valinomycin-alkali metal ion complexes in ethanol

| | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | $Ca^{++}$ |
|---|---|---|---|---|---|---|
| Log K[a] | — | — | 6.3 | 6.46 | 5.81 | — |
| Rel. Log K | — | — | 5.0 | 5.12 | 4.61 | — |
| Rel. Intensity[b] | — | 1.0 | 4.7 | 12.9 | 9.9 | 0.19 |
| Rel. Intensity[c] | 0.21 | 1.0 | 5.2 | 13.6 | 10.5 | 0.18 |

[a] Measured by conductivity method.[14]
[b] The concentrations of metal ions and **1** are $3.46 \times 10^{-4}$ M and $1.73 \times 10^{-5}$ M. ESI chamber temperature 50 °C; needle voltage 2.46 kV.
[c] The concentrations of metal ions and **1** are $3.6 \times 10^{-4}$ M and $9 \times 10^{-6}$ M. ESI parameters are same as in [b].

**Table 3** Potassium ion affinities of valinomycin, 18-crown-6 and [2,2,2]-cryptate in methanol

| | *valinomycin* | *18-crown-6* | *[2,2,2]-cryptate* |
|---|---|---|---|
| Log K[a] | 4.90[b] | 6.18[c] | 10.8[d] |
| Rel. Intensity[e] | >80 | 1.0 | 3.0 |

[a] Reference 14.
[b] Spectrophotometric method.
[c] Potentiometric method.
[d] In $CH_3OH$ with 0.05 M $Et_4NClO_4$ by potentiometric method.
[e] The concentrations of KOAc and ligands are $8.46 \times 10^{-4}$ M and $8.46 \times 10^{-6}$ M. The intensities of the complexes of **1** are the sum of peaks 3, 4 and 5.

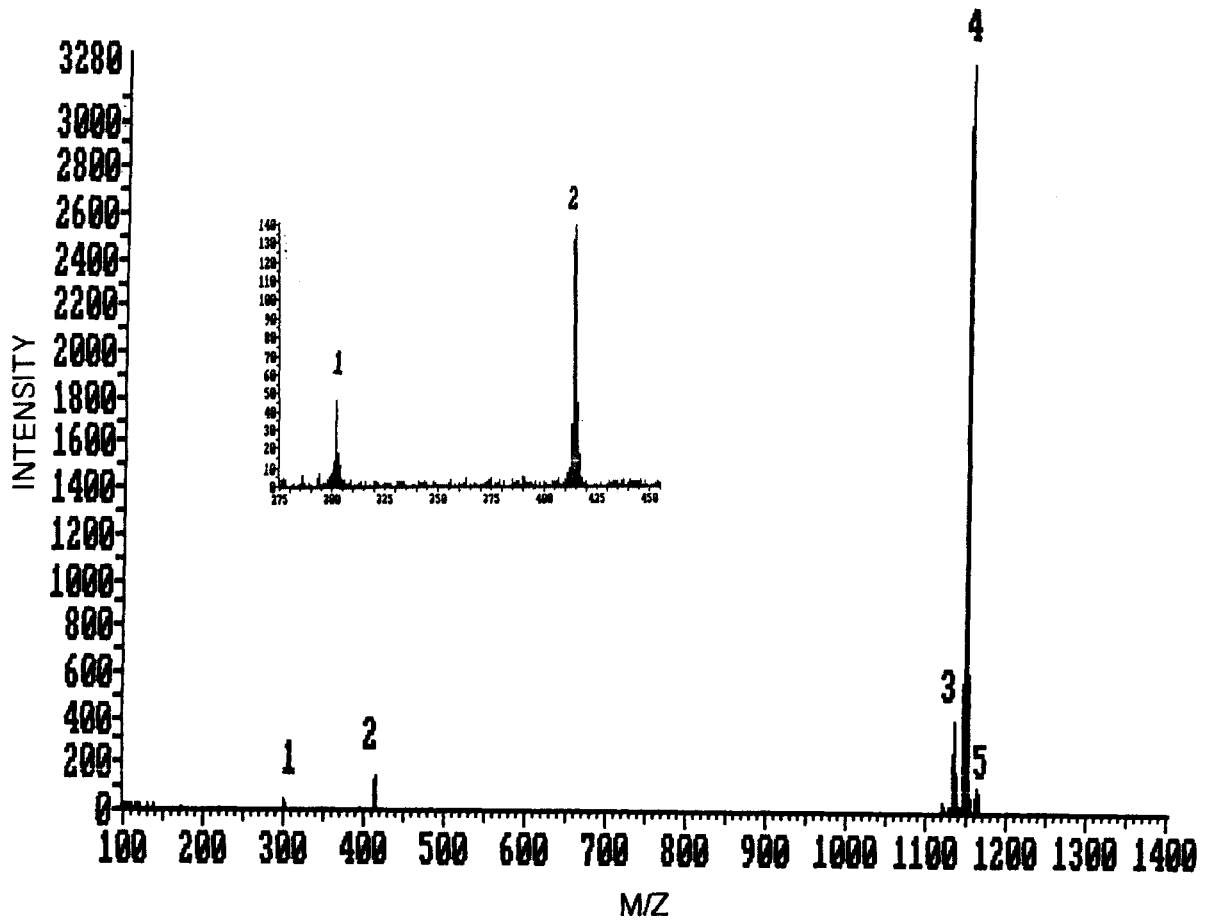



**Figure 2** ESI-MS spectrum of valinomycin, 18-crown-6 (2) and [2,2,2]-cryptate in the presence of excess potassium acetate (1:2:3:$K^+$ = 1:1:1:100). Peak assignments are: 1. $[2 + K^+]$, 2. $[3 + K]^+$, 3. $[1 + Na]^+$, 4. $[1 + K]^+$, 5. $[1 + Na + CH_3OH]^+$. Inset shows an expansion of the region 275–450 m/z.

$K^+$ complexes in methanol. Valinomycin gave as much as 80 times higher intensity than 18-crown-6, very poor correlation with the data from condensed phase.

We have investigated the effect of ESI repeller voltage (an instrument parameter affecting fragmentation) on the ion intensity for a mixture of ions. A parallel (and small) change in metal complex signal intensity was observed, implying that the change of repeller voltage has small effect in this case (Fig 3).

From the above results, it is clear that the correlation between observed complex intensities and their solution binding constants of different compound classes are not straightforward. We think both solutions and gas phase ion chemistry are involved. When complexes of one ligand with several guests are compared, gas phase chemistry seems less important. In some cases such as for $K^+$ and $Na^+$, the ESI-MS responses of their complexes with valinomycin are in good agreement with the solution binding constants, even in the presence of other metal ions. But the complexes with larger guests $Rb^+$ and $Cs^+$ gave much stronger signals than expected from solution chemistry.

Based on solution binding constants, one would expect competition in solution between valinomycin (**1**), 18-crown (**2**) and [2,2,2]-cryptate (**3**) to lead to large concentration of **3** + $K^+$ and smaller concentration of **1** + $K^+$. In fact, the opposite order from ion intensities was observed. A referee suggested that one possible explanation is that the high polarizability of valinomycin may lead to a faster gas-phase $K^+$ attachment reaction via ion transfer process than the other two. It is clear that valinomycin and the crown ether type compounds behave differently in ESI-MS and 18-crown-6 **2** and [2,2,2]-cryptate **3** may not be good models for valinomycin under ESI-MS conditions. Relative binding of similar types (in size, structure) of compounds is better for ESI-MS.[5]

During this study, we have found that an unusually strong signal for the $Na^+$ complex was observed when the metal ion concentrations are very low (about $10^{-6}$ M). With the increase of metal ion concentrations (i.e. large excess alkali salts), the relative intensities of complexes became almost independent of the salt concentrations. It has been reported that the smaller cation ($Na^+$) attaches more rapidly in the gas phase to the ligand than larger and less charge-dense cations.[15] Thus $Na^+$ interferes with ESI-MS measurements at low ion concentrations.

The observation of solvated metal complexes was first reported by Chait.[16] In our ESI-MS study on Ni (II)-bipyridyl complexes, similar type of species were detected in acetonitrile.[ref] In protic solvents like water and methanol, solvated molecular ions are seen sometimes, presumably via hydrogen binding to the target molecule. On our instrument, the intensities of solvated ions are dependent on several factors such as repeller voltage, ESOI chamber temperature and block temperature.

It is unclear why $Ca^{++}$ only gave very weak signals although it has similar size to $Na^+$. During an ESI-MS study on Cu (II)-peptide complexes, we obtained clean spectra in which ions corresponded to singly charged complexes of Cu (II) and the deprotonated peptides, i.e. [peptide $- H^+ + Cu^{++}]^+$.[17] We thought $Ca^{++}$ may form similar type of complex with **1**, however, only the doubly charged complex was observed. Even in the mixture study, the presence of $Ca^{++}$ did not affect the relative intensity of **1** + $K^+$ which has nearly the same mass as [**1** $- H^+ + Ca^{++}]^+$ and is unlikely to be differentiated by our instrument.

## EXPERIMENTAL

### General

Valinomycin was purchased from Sigma Chemical Company, Inc. 18-crown-6 and [2,2,2]-cryptate were purchased from Aldrich Chemical Company, Inc. HPLC grade methanol was from Fisher Scientific. Absolute ethanol (200 proof dehydrated, U.S.P.) was from Quantum Chemical Corporation.

General procedure for sample preparation: A standard solution of each ligand (**1**, **2** or **3**) and each salt in alcohol was prepared. Calculated aliquots were mixed and after standing at rt for about 30 minutes, the mixture was infused into the ESI mass spectrometer as described below.

For example, 0.2 ml of **1** ($1.1 \times 10^{-4}$ M in $CH_3OH$), 0.2 ml of **2** ($1.1 \times 10^{-4}$ M in $CH_3OH$), 0.2 ml of **3** ($1.1 \times 10^{-4}$ M in $CH_3OH$) and 2 ml of KOAc ($1.1 \times 10^{-3}$ M in $CH_3OH$) were mixed to generate the sample for experiment corresponding to the result in Table 3.

Electrospray mass spectra were obtained on a Vestec Model 201 single-quadrupole mass spectrometer.[18] Sample solutions were infused directly into the ESI source by a syringe pump (Sage Instrument). General instrumental settings, unless specified, are as follows: needle voltage: 2.0–2.4 kV; flow rate: 5 $\mu$l/min; electrospray chamber temperature: 45–60 °C; nozzle voltage: 200 V; block temperature: 250 °C; lens temperature: 120 °C; repeller voltage: 20 V.

The relative intensities in all tables were obtained by averaging the spectra over 2–3 minutes (101–20 scans) after obtaining a stable ion current.

## CONCLUSION

We have demonstrated that ESI-MS is a very good

tool for examination of solution complexes involving metal ions because of its sensitivity and mild ionization. We have shown that this *solution* MS technique provides a nice complement to gas phase methods. There are no other techniques available for *simultaneous determination* of binding in solution of up to 6 metal ions! With a full understanding of the electrospray process and standardization, quantitative measurement of binding constant should be feasible with related compounds.

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